

THE SPERMATOOZON AND FERTILIZATION MEMBRANE OF ARBACIA PUNCTULATA AS SHOWN BY THE ELECTRON MICROSCOPE¹

ETHEL BROWNE HARVEY AND THOMAS F. ANDERSON²

(The Marine Biological Laboratory, Woods Hole; the Biological Laboratory, Princeton University; and the Eldridge Reeves Johnson Foundation for Medical Physics, University of Pennsylvania)

Spermatozoa have been studied for many years with the light microscope, and the general structure of many kinds of spermatozoa has been described. This study of the spermatozoa of *Arbacia punctulata* was undertaken to throw further light on their structure by the use of the electron microscope.

The fertilization membrane of the *Arbacia* egg which is thrown off two minutes after fertilization is now generally believed to have been, at least in part, the plasma (or cell) membrane before fertilization. The fertilization membrane was therefore studied in the hope that the electron microscope would throw some light on the structure of the plasma membrane.

TECHNIQUE

The preparation of various kinds of biological material for the electron microscope has already been described in some detail (see Anderson, 1942, and references given therein). Briefly the procedure involves: (1) the complete removal of the sea water by washing several times with distilled water, in order to avoid the formation of salt crystals; (2) placing the material on a thin collodion membrane across a fine mesh wire screen (200 mesh per inch); (3) allowing it to dry; and (4) placing the screen in the electron microscope. In the present work an "RCA type B" microscope was used and the micrographs were taken with 60 kilovolt electrons.

The *Arbacia* sperm were taken directly from the testis of a freshly opened animal and diluted in sea water. They were then mounted on the collodion membrane to which they adhered, washed in several changes of distilled water, and then dried.

The preparation of the fertilization membranes presented greater technical difficulties; since they seemed to show no tendency to adhere to the collodion membranes, they had to be freed from the eggs and washed before they could be placed on the specimen screens. The fertilization membranes are formed about two minutes after fertilization of the eggs in sea water at 23° C. It was found that if the eggs are placed in distilled water one minute after formation of the fertilization membranes, these rupture and the egg contents flow out, leaving the empty membranes. If placed in distilled water a minute or two later, only part of the contents come out, and still later none at all. The procedure of washing

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² Formerly RCA Fellow of the National Research Council.

the eggs several times in distilled water three minutes after fertilization was therefore adopted for separating the membranes from egg material and freeing them from salt. Under these conditions, the empty fertilization membranes sometimes retain their spherical shape, but usually collapse and become crinkled; they settle more slowly than the egg material to form a layer just above the bottom of the dish where the eggs lie. With a micropipette, under a binocular dissecting microscope, a number of membranes were taken up and deposited in tiny drops at the centers of the collodion membranes. The specimens were then dried in air and studied in the electron microscope.

RESULTS AND DISCUSSION

Arbacia spermatozoon

The *Arbacia* spermatozoon at high magnification with the light microscope (Fig. 1) is observed to possess a pointed head with a flattened base adjacent to a short, slightly narrower, middle piece which seems to contain a pair of spherical bodies. The long thin filamentous tail extends from the middle piece. The head (with middle piece) measures approximately $4\ \mu$ long and $2\ \mu$ across the base; the tail is approximately $45\ \mu$ long. When placed in distilled water, the heads were observed to swell to about twice their original size.

In the electron microscope, the changes in structure caused by washing and drying are immediately apparent (Figs. 2 to 4). In most cases (except Figure 3), the heads have lost their characteristic arrow-head shape, and material appears to be flowing out of them. There is no distinct middle piece. The tails are, in most cases, coiled around the heads and consist of strands; the ends resemble frayed ends of rope unwrapped into separate strands (Fig. 2). The strands themselves are frequently detached, broken up, and strewn about the field (Fig. 3).

When examined more closely under higher magnification (Figs. 3, 4), a number of interesting features are apparent in the tails. Each tail appears to have been made up of about ten strands of uniform thickness, each having a diameter of about $50\ m\mu$. In some of the micrographs, the tail has the appearance of a thick core surrounded by a sheath, but this appearance might be produced by a number of fibrils being superimposed at the center and flanked by one or two single fibrils. Occasionally one sees individual fibrils apparently broken up into short rods lined up in a row (Fig. 3), but this may be an artifact produced by drying, shrinking, and breaking. The regularly spaced cross striations which appear along the tail in certain areas (Fig. 4) may be characteristic of the material as has been reported for collagen fibers (Schmitt, Hall and Jakus, 1942) or may be an artifact of drying analogous to the formation of the rods noted above, but on a smaller scale.

PLATE I

FIGURE 1. Living spermatozoa of *Arbacia punctulata* as photographed with the light microscope. $\times 1,000$.

FIGURE 2. Spermatozoa of *Arbacia punctulata* micrographed with the electron microscope showing the appearance after washing in distilled water and drying. $\times 2,200$.

FIGURE 3. Head and fragments of the tail of a spermatozoon at high magnification with the electron microscope. $\times 15,000$.



PLATE I

Unfortunately, the heads are too thick to show much internal structure. In some of the micrographs, one sees a small round area of low density which might represent a vacuole. There is also, in one of the micrographs (Fig. 4), a lighter area of the head having the appearance of a membrane. This is interpreted as the outer membrane left more or less intact on drying while the material inside has withdrawn and flowed out at the sides. It is not possible to determine the structure of the nuclear material from these micrographs. Some of the material found in the neighborhood of the heads appears to have interesting structure, such as the small rings, but it is impossible to identify it at this time.

Fertilization membrane of Arbacia

In the light microscope, the fertilization membrane of *Arbacia punctulata* appears as a uniformly thin and transparent membrane 3 to 5 μ from the surface of the egg. It is quite elastic when first formed, as shown by the fact that in high centrifugal fields it stretches from a sphere having a diameter of 80 μ to a spheroid having a length of 140 μ (Harvey, 1933, and unpublished observations). Five minutes after fertilization, however, the membrane thickens and hardens and resists stretching. Membranes freed from the eggs one minute after fertilization in distilled water have been observed to last 12 hours without any apparent change.

A number of electron micrographs of various fertilization membranes were taken and none showed anything but a thin amorphous structure (the membrane) sprinkled with what appears to be débris (Fig. 5). This débris may actually represent the structure of certain components of the cell or plasma membrane of the unfertilized egg, but the fact that they are neither characteristic in shape nor distributed in definite patterns on the surface prohibits one from attaching any special significance to them. There are no pores of sufficient size to be recognizable as such in the micrographs. From the apparent density of the micrograph one can estimate the thickness of the fertilization membrane, when first formed and dried, to be of the order of 25 $m\mu$. It is of interest to note that this estimate is approximately the same as that of the membrane of the red blood cell. In the recent work of Zwickau (1941), who studied the red cell membranes with an electron microscope, the thickness of the membrane of the dried ghost is given as 20–30 $m\mu$. Other estimates of the thickness of the intact red blood cell membrane, including water and diffusible proteins range from 20 $m\mu$ to as much as 50 $m\mu$ (see Ponder, 1942). The electron micrographs of the red blood cell membranes given by Zwickau show no definite structures.

PLATE II

FIGURE 4. Spermatozoon, disrupted by distilled water, showing the multiple stranded structure of the tail with cross striations, and the remains of what may have been the membrane of the head—with the electron microscope. $\times 10,000$.

FIGURE 5. Electron micrograph of the fertilization membrane of an *Arbacia punctulata* egg. At the top of the field is the collodion film on which the specimen is mounted with a hole in it at the upper left hand corner. The fertilization membrane comes up from the bottom of the field and folds over on itself near the top. The dark line extending from the upper left hand corner is a wrinkle in the film. Note the frayed edge of the fertilization membrane to the left of the middle of this wrinkle. $\times 22,000$.

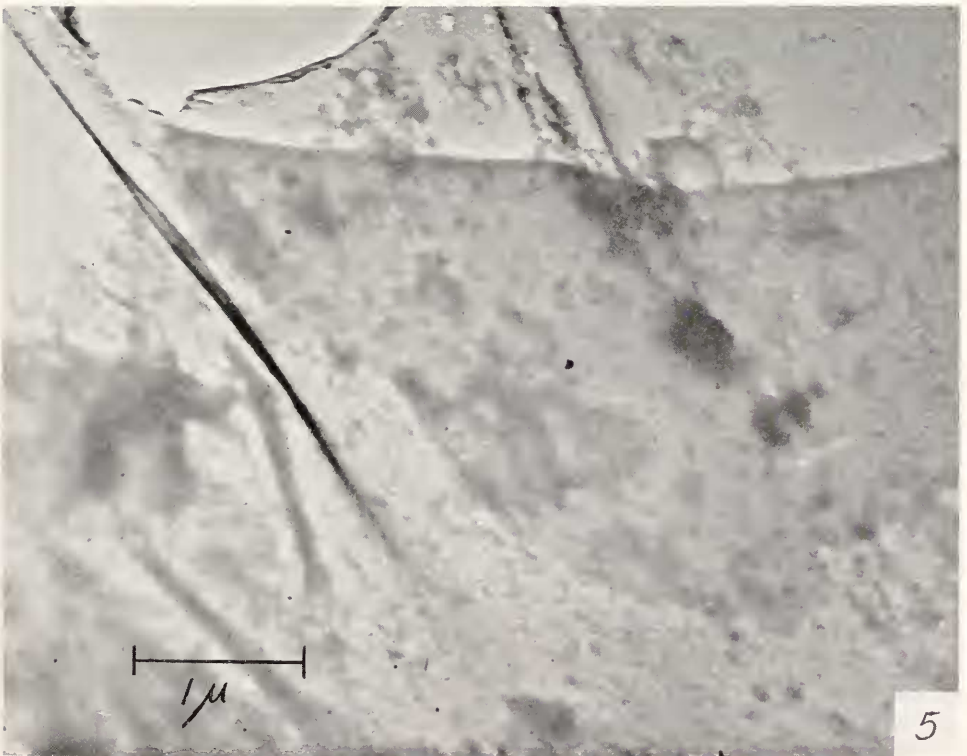


PLATE II

SUMMARY

1. As studied with the electron microscope, the tail of the *Arbacia punctulata* spermatozoon is found to disrupt into about ten distinct fibrils when it is washed in distilled water and dried. Each fibril is about 50 $m\mu$ in thickness. Regularly spaced cross striations also appear in the tail structure, but these may be produced in the washing and drying process.

2. A method of obtaining the fertilization membranes of *Arbacia punctulata* eggs free from egg material is described. When these were washed in distilled water and dried for examination, the electron microscope revealed no regular structures nor definite patterns. The thickness of the fertilization membrane, when first formed and dried is estimated to be of the order of 25 $m\mu$.

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